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Effect of Virulence Aspergillus Fumigatus Isolate in Liver of BALB/C Mice

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Abstract

Aspergillosis is considered one of the dangerous systemic fungal infections which develop mainly in immunocompromised individuals. The most common cause is Aspergillus fumigatus. The present study aimed to investigate the Histopathological changes of Aspergillus fumigatus in albino mice were obtained from the current study :Histopathological changes in liver, of BALB/c mice at the first 10 days of treatment with spore supention of pathogenic Aspergillus fumigatus in addition to sever pathological symptoms appeared on mice treated with the same period. Histopathologic changes of liver after 10 day from infections with Aspergillus fumigatus represented by sever Congestion, Inflammatory cells while in the liver after 20 day from infection included necrosis hepatocyte cell, inflammatory cell and expansion of sinusoids .

Keywords: fungi; Aspergillus fumigatus; histopathological changes.

1. Introduction

Hepatic aspergillosis may occur as a process of dissemination from the gastrointestinal tract along the portal venous system or as a component general systemic dissemination.

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It may also develop as a process of cholangitis reports of therapeutic interventions are limited [1]. Primary hepatic invasive aspergillosis (IA) may progresses under anti-CD20 B cell treatment for post transplantation lymph proliferative disease following allogeneic stem cell transplantation [2,3]. Medical therapy for hepatic abscesses may be effective and preclude the need for surgical resection [4].

Histological changes that found in the liver of Aspergillus fumigatus infected mice in comparison to control group including: damages of hepatic cells with necrosis, moreover lymphocytes infiltration and hemorrhage especially near portal space [5]. But the infected camels showed massive areas of haemorrhage [6]. Also Nejak-Bowen et al., [7] show treated rats with A.fumigatus, mitosis was absent and apoptotic Hepatic non-parenchymal cells (NPCs) were apparent.

Aims of the study: The main objective of this study was to identify the changes in liver tissue during infection with A.fumigatus.

2. Material and Methods

Preparation of spore suspension for Aspergillus fumigatus isolates

After the isolates were cultured on sabouraud dextrose agar at 37 °C for 7 days, fungal colonies were covered with 10 ml of sterile saline solution for spore suspension preparing, and the suspensions were prepared by gently agitation of the surface with the tip of a pasture pipette. The spore suspension was filtered through sterile gauze , and then the filtration was transferred to a sterile test tube. Inoculums quantification was made by counting the spores using haemocytometer by added one drop of the suspension to haemocytometer by Pasteur pipette, spores were calculated under high power 40X of light microscope using the following equation:

Concentration of spores = (Z *4 * 106) /n spores/ml.

Where n: total No. of small squares Z: total No. of spores [8].

A total number of 20 Albino mice of male with ages ranged (6–8) weeks old which were obtained from the (National Center of Researches and Drugs Monitor in Baghdad) were adapted for two weeks in Biotechnology Research Center/ Al Nahrain University before started experiment by rearing in separated, cleaned and disinfected cages, they were fed on commercial assorted pellets and tap water.

The group (n=15) the mice were inoculated intranasally with 5*106 conidia in 20 -0.01% PBS Tween 20.
(Five mice per group) of the BALB/c strain Control mice were inoculated intranasally with 20 -0.01% PBS Tween 20. Mice were sacrificed after 10 and 20 days post infection.

Histopathological sections of the lungs of BALB/c strain were prepared from each mice treated and from control and used for comparisons among the groups , Pieces from lunge were fixed in 10% formalin for 1 day cut at 5 µm thickness (Mantovani, 1978). For histopathological studies this experiment were done in the Pathology Department/ veterinary collage/ Baghdad University, Baghdad, Iraq. Histopathological section were prepared

according to the following procedure [9].

3. Statistical analysis

Complete Randomized Design (C.R.D.) was used as an experimental design. Data were analyzed using SAS [10] to study the effect of different factors on the diameters of inhibition zones. Least significant difference (LSD) was used to compare the significant difference between means at $P \leq 0.05$.

4. Results and Discussion

Histological examination of the control liver expressed normal structure show no clear lesions (H&E stain40X), as showed in figure 1.

Histopathological examination of liver of mouse appear signifies difference $p \geq 0.05$ and the effect mice treated with spore suspension of A.fumigatus was increase with the increase of the exposure date, after 10 days of infections showed granulomatous lesion consisting from aggregation of macrophages and lymphocytes and expressed cells division with dark chromatin of hepatocytes or disappear of their nuclei (figure 2). Another field appeared necrotic area in their parenchyma.

The main lesions in the liver after 10 day of infection consisting from multiple granuloma with proliferation of kupffer cells also necrotic of hepatocyte characterized by pyknotic nuclei or disappearing.

A granulomatous lesion with proliferation of kupffer cells (figure 2) were shown in liver of animal treated with spore suspention after 20 days from infection

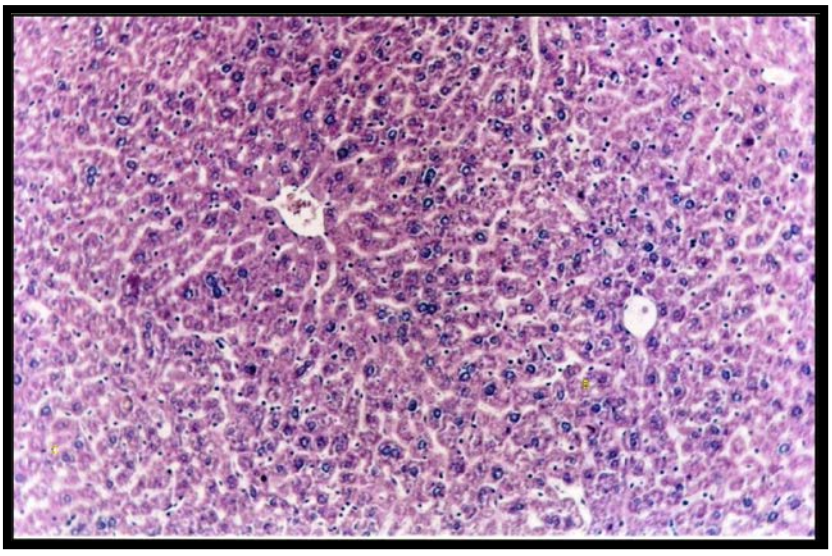

Figure 1: Histological section in the normal liver of albino mouse shows no clear lesions (H&E stain, 40X)

was recorded aggregation of mononuclear cells in liver parenchyma, vacuolar degeneration, necrosis and proliferation of kupffer cells , other field showed cell division, vacuolar degeneration and necrosis of

hepatocytes also revealed inflammatory cells infiltration particularly mononuclear cells in their capsular region and showed granulomatous lesion, vacuolar degeneration, mononuclear cells and megakerocytes in sinusoids with proliferation of kupffer cells as well as mild fatty changes.

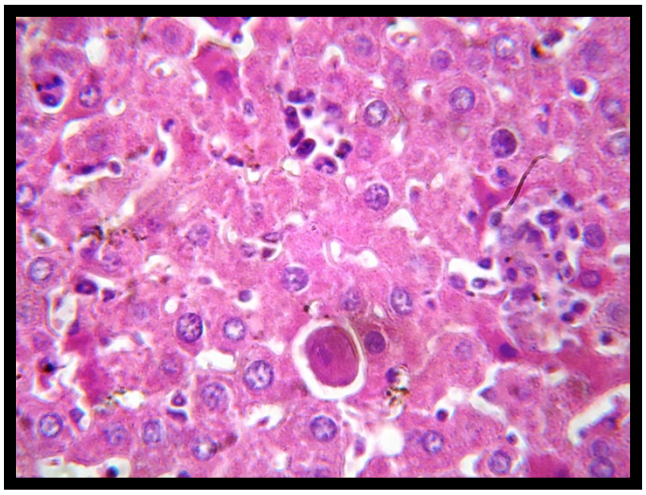

Figure 2: Histopathoogical section in the liver of mouse treated with pathogenic Aspergillus fumigatus after 10 day of infection shows aggregation of mononuclear cells and vacuolar degeneration ,necrosis and proliferation of kupffer cells 2 (H&E stain40X).

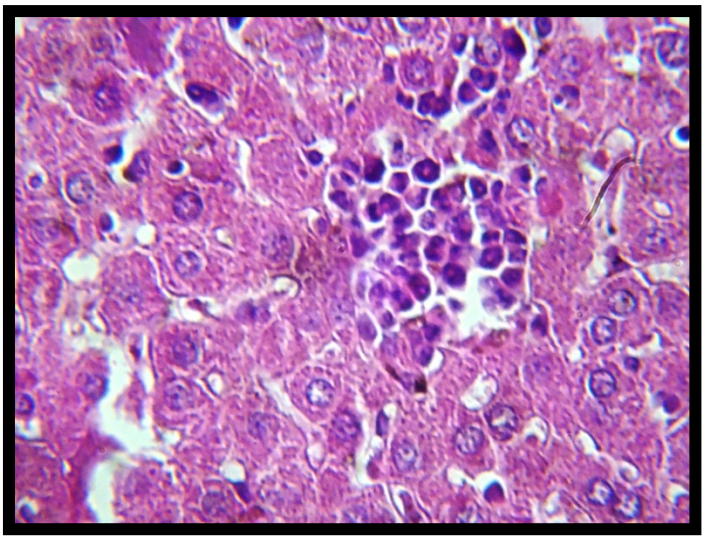

Figure 3: Histopathoogical section in the liver of mouse treated with pathogenic Aspergillus fumigatus shows multiple granulomas with proliferation of kupffer cells (H&E stain40X).

Pathogenicity of A.fumigatus in animals and human related to its ability to produce different virulence factor was shown in many studies; Shafiq and Al-Joofy, [11]found histological changes in the liver of infected mice in comparison to control group including: damages of hepatic cells with necrosis, moreover lymphocytes infiltration and hemorrhage especially near portal space. The infected camels showed massive areas of

haemorrhage [12]. In A.fumigatus treated rats, mitosis was absent, hepatocyte growth factor (HGF) was decreased and apoptotic Hepatic non-parenchymal cells (NPCs) were apparent [7].

The innate host defense system (IHDS) against *A. fumigatus* includes dedicated phagocytic cells (peripheral blood monocytes, monocyte derived macrophages, pulmonary alveolar macrophages, neutrophils, myeloid dendritic cells and natural killer cells) *A. fiumigatus* is reported to produce endotoxins, fumitremorgins and mycotoxins such as fumagillin, fumagatin, gliotoxin, and helvolic acid which may be significant in disease processes. It has also been speculated that proteases play a role in pathogenesis (These secondary metabolites have the potential to significantly influence the response of endothelial cells to fungal infection [13,14].

5. Conclusions and Recommendations

Different histopathological changes in mice were shown by the AFU1 isolate we recommended the use of more accurate, faster and easier methods for detection of virulence factors in others pathogenic fungal isolates using molecular technique.

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